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Neuronal Nicotinic Acetylcholine Receptors: The Development of Methods for Producing Affinity-Purified and Lipid-Reconstituted Receptors that Retain Functionality

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Neuronal nicotinic acetylcholine receptors (nAChRs) are allosteric proteins which belong to the Cys-loop superfamily of pentameric ligand-gated ion channels. Many therapeutically relevant drugs act as positive/negative allosteric modulators of these receptors and are sensitive/selective to the conformational state of the receptor. Studies to identify the molecular interactions of an agent with a particular neuronal nAChR via a technique such as photoaffinity labeling require purified receptors that retain the ability to undergo conformational transitions between functional states. However, producing purified neuronal nAChRs that are fully functional has proven to be a challenge (see Ref 1-2). With the present study we are systematically assessing the functionality of neuronal nAChRs at different critical steps during isolation (membrane isolation, detergent solubilization, lipid reconstitution, etc). Each step is optimized with respect to both increasing the yield of protein, but importantly also to ascertain functionality. Pre-assembled nAChRs are microinjected into oocytes, a method pioneered by the Miledi group (3), and following fusion of receptors with the oocyte plasma-membrane, two-electrode voltage-clamp recordings are used to monitor receptor functionality.

[1] Hamouda et al (2007) *Biochemistry* 46(48), 13837-46.
[2] daCosta et al (2011) *Biochem Biophys Res Commun* 407(3), 456-60.
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Stoichiometry for Activation of Neuronal α7 Nicotinic Receptors

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Neuronal α7 nicotinic receptors elicit rapid calcium influx in response to acetylcholine (ACh) or its product choline. They contribute to cognition, synaptic plasticity, and neuroprotection, and have been implicated in neurodegenerative and neuropsychiatric disorders. α7, however, often localizes distal to sites of nerve-released ACh, binds ACh with low affinity, and thus elicits its biological response with low agonist occupancy. To assess function of α7 when neurotransmitter occupies fewer than five of its identical binding sites, we measured open-channel lifetime of individual receptors in which four of the five ACh binding-sites were disabled. We find that in receptors with only one intact binding site, open-channel lifetime is indistinguishable from receptors with five intact binding sites, counter to expectations from prototypical neurotransmitter-gated ion channels where open-channel lifetime increases with the number of binding sites occupied by agonist. The replacement of the membrane-embedded domain of α7 by that of the related 5-HT_3A receptor changes the dependence of open-channel stability on ACh occupancy, thus revealing a novel interdependence between the detector and actuator domains of these receptors. The distinctive ability of a single occupancy to elicit a full biological response adapts α7 to volume transmission, a prevalent mechanism of ACh-mediated signaling in the nervous system and non-neuronal cells.

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Progresive Analysis of nAChR Stability in the Lipidic Cubic Phase: nAChR Detergent Solubilization and Fractional Mobility

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Nicotinic acetylcholine receptors (nAChRs) are transmembrane receptors, members of the ligand-gated ion channels (LGIC) family, and key component of the neuromuscular junction. On vertebrate they mediate nervous impulse supporting the synaptic mechanism. As a result the nAChR are important targets for the treatment of neurodegenerative diseases such as Alzheimer's, Parkinson's, epilepsy, and schizophrenia (Albequerque et al. 2009)., as well as cardiovascular disease, inflammation (Wang et al 2003), depression, and nicotine addiction. On the past four decades the nAChR have being extensively study, however the relation between detergent solubilization and nAChR stability is still poorly understood. Our work considers the detergent structural features and physical properties to classify the detergents into cholesterol-analogs and phospholipid-analogs; which will be employed to isolates nAChR from Torpedo californica's electroplax tissue. The present study attempt to better understand how detergent solubilization affects the nAChR stability once inserted to the Lipidic Cubic Phase (LCP). We examined the Fractional Mobility (FM) of the nAChR on the LCP through fluorescence recovery after photo bleaching (FRAP) experiments for a 30 days period. Our results show that phospholipid-analog detergents with 16 carbon acyl chains sustain nAChR mobility unchanged for the 30 day period, while in most of cholesterol-analog detergents, the nAChR moility decays on the first 15 to 20 days of the experiment. In addition there is an interesting correlation between the elongation of the acyl chain on phospholipid-analog detergents and an increment on the FM, particularly observed on the Lyso-Foscholine detergent family. This work is supported by NIH Grant 1R01GM098343-01. Luis F. Padilla-Morales and Joel E. González Nieves equally contribute to this work and both will be presenters.

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Calculation of Cholesterol Binding Affinity for Pentameric Ligand-Gated Ion Channels

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Cholesterol has been shown to play a critical role in the function of ion channels and many types of ion channels partition into cholesterol-rich membranes. Several Eukaryotic pentameric ligand-gated ion channels, including the nicotinic Acetylcholine Receptor and the GABA(A) receptor, are highly sensitive to the cholesterol content of reconstitution mixtures or native membranes, but the binding interactions between cholesterol and these receptors are largely uncharacterized. Since the mechanisms for modulation by cholesterol are likely to be at least partially through direct interactions via specific binding, cholesterol molecules may form an essential component of the native structure for some of these receptors. We recently proposed, based on the crystal structure of the glutamate-gated chloride channel (GluCl) from C. elegans solved in complex with the lipophilic ligand ivermectin, that cholesterol molecules could bind to these intersubunit sites in a pose analogous to that of ivermectin in the absence of other modulators, and tested this model in the homologous GABA(A) receptor using Molecular Dynamics Simulations. Here we employ computational free energy calculation methods to provide a quantitative estimate of the binding affinity of cholesterol for these sites on GluCl and other pentameric ligand-gated ion channels, demonstrating that these sites are likely to be occupied at sufficiently high concentrations of cholesterol.

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Agonist Response Induced by Nicotinic α-7 Agonist is Inhibited by Antipsychotic Drugs

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Nicotinic acetylcholine α7 receptors (nAChRα7) have been implicated in certain pathological conditions associated with memory impairment, including schizophrenia and Alzheimer's disease. In vivo experiments have shown that both nAChRα7 agonist and nAChRα7 positive allosteric modulators (PAMs) can improve cognition in various preclinical models. Following these results there has been a lot of interest in developing new nAChRα7 ligands for the potential use for cognition in both schizophrenia and Alzheimer's disease. The use of new nAChRα7 compounds in schizophrenia would probably be add-on to existing antipsychotics. Therefore it is important to investigate if antipsychotic compounds have any effects on the positive effects of new nAChRα7 compounds. We have previously shown that antipsychotic compounds are inhibitors of ACh induced responses at h-α7 receptors with IC_{50} values between 2100 and 6500 nM. In this study we have further evaluated the effects of antipsychotic compounds on the agonistic response at h-α7 receptors induced by nAChRα7 agonists i.e. EVP-6124 (EVP) and TC-5619 (TC). All tested antipsychotics compounds showed inhibition of the agonistic response induced by both EVP and TC at h-α7 receptors with the following IC_{50} values in nM for EVP and TC, respectively: haloperidol (2400, 1900), clozapine (1800, 2700), olanzapine (3000, 2000), quetiapine (3200, 3000), aripiprazole (2300, 1900) and risperidone (2000, 1500). These IC_{50} values are higher than the clinically plasma concentrations for antipsychotics drugs in stabile out patients. In summary, it was shown that antipsychotic compounds antagonize both EVP-6124 and TC-5619 induced agonistic response at nAChRα7 receptors with IC_{50} values between 1500 and 3200 nM.